REFEYN
WEIGHING MOLECULES WITH LIGHT

Refeyn Acquire^{MP} User Manual

Version 2023 R1.1

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Introduction

Refeyn Acquire^{MP} is the control software for all Refeyn Mass Photometers. The software facilitates recording movies on a Mass Photometer, and tuning operating parameters of the instrument to optimise the recording and measurement protocol.

Getting started

Compatibility

Device

This version of Acquire^{MP} is compatible with the following devices:

- Refeyn One^{MP}
- Refeyn Two^{MP}
- Refeyn One^{MP} Auto
- Refeyn Two^{MP} Auto
- Refeyn Samux^{MP}
- Refeyn Samux^{MP} Auto

Discover^{MP}

Movies (.mp files) acquired in Acquire^{MP} version 2023 R1 can only be analysed in Discover^{MP} version 2023 R1 or later.

Prerequisites

System requirements

The required PC specification for Acquire^{MP} depends on the instrument it is being used with.

One^{MP} and Two^{MP}

- PC
  - CPU: Intel i7 with 8 cores (e.g. Intel i7-10700)
  - Memory: 16 GB DDR4 RAM
  - Storage: 1TB SSD
- Monitor: 1920 x 1080 (e.g. HP EliteDisplay E24 G4)
- Operating System: Windows 10 Professional

Samux^{MP}, One^{MP} Auto, Two^{MP} Auto and Samux^{MP} Auto

- PC
  - CPU: Intel i9 with 18 cores (e.g. Intel i9-10980XE)
  - Memory: 16 GB DDR4 RAM
  - Storage: 1TB SSD
- Monitor: 1920 x 1080 (e.g. HP EliteDisplay E24 G4)
- Operating System: Windows 10 Professional

Check drivers and connections

Before starting Acquire^{MP} the required connections between the PC and instrument hardware should be checked.

- Check that the instrument is powered.
- Ensure that the Universal Serial Bus (USB) cable from the instrument is connected to one of the USB 3 ports of the Personal Computer (PC). (labelled with the symbol SS⇐)
- Open the Control Panel > Devices and Printers and check that the following devices in the instrument are correctly recognised:
  - "Digilent USB Device"
  - "Grasshopper3" or "xiC"
  - "uc480 cmos" or "USB 2.0 Camera" or "xiQ"
  - "SmarAct MCS2"
  - "USB-3103" [One^{MP} only]

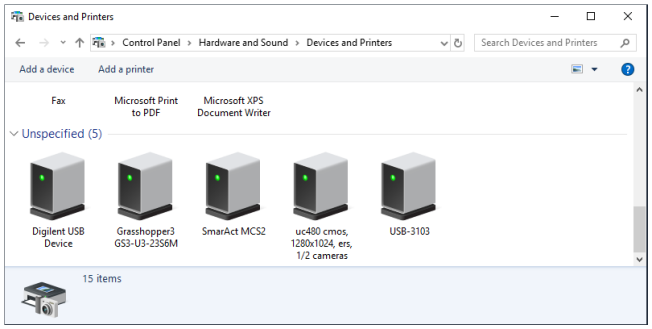


Figure 1: List of device names shown in the control panel when the One^{MP} is turned on and correctly connected

If any device is missing:

1) Power-cycle the USB devices by disconnecting and reconnecting the USB cable.
2) Make sure that the correct camera drivers are in use.

Check DAC registry (One^{MP} and One^{MP} Auto only)

The One^{MP}'s Digital-to-analog converter (DAC) card may require its registry to be updated when Acquire^{MP} is run for the first time or if there is a Windows update on the PC. To update the registry, start the program **Instacal** which will then show a prompt to update the registry. Once the update is complete either the PC will need to be restarted or the USB connection of the mass photometer needs to be cycled by removing and plugging in the USB cable to the PC.

Installing/updating

If installing Acquire^{MP} for the first time, jump to step 4.

1. Ensure that all instances of Acquire^{MP} are closed.
2. Uninstall the old version of Acquire^{MP} by going to Control Panel > Programs > Programs and Features and then selecting Acquire^{MP} to uninstall.

3. Check that the uninstall has been successful by going to C:\Program Files (x86) in the windows file explorer and checking that there is no folder called AcquireMP. If there is, manually delete the folder.
4. Execute the Acquire^{MP} setup executable file (the filename will be of the form: AcquireMP_v2023.1.000_setup.exe). Double clicking on the file will bring up an install wizard.
5. Follow the steps of the installer, ensure that the terms and conditions have been read before accepting.
6. Once the installer has finished, the software is ready to use.

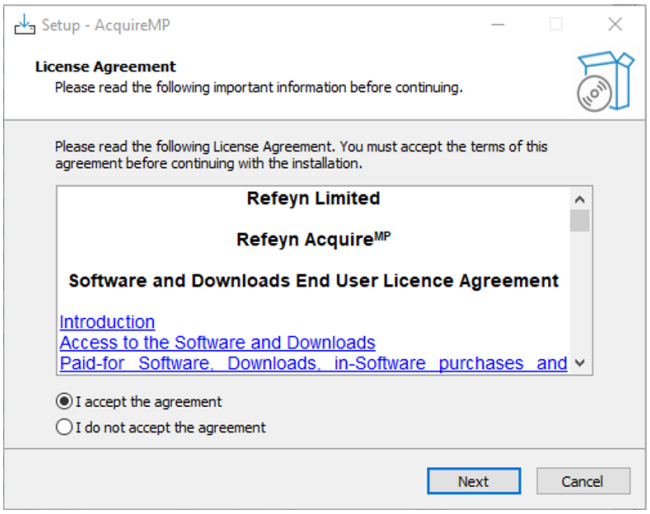


Figure 2: Installation dialog

Licensing

When opening the application for the first time, the program will prompt you to activate the license with the license key. The license key is provided with the software, and appears such as the following example: ABCDE-FGHIJ-KLMNO-PQRST. If the user has yet to obtain a license key, one can be obtained by contacting Refeyn using the contact details.

If the PC on which you are installing Acquire^{MP} has an internet connection, make sure **Use this PC's internet connection** is selected, then simply enter the license key into the box and click **OK**.

If the PC does not have an internet connection or the connection fails, select **Use another PC's internet connection**. The license key code is then to be entered into the box, click **Next** and take note of the *machine code* shown in the dialog. If access is necessary on an additional PC with an internet connection, open the webpage shown in the dialog. A form will be loaded in which the machine code and license key shall be entered. On clicking **Activate**, an .skm activation file will be downloaded. Copy this file onto the desired PC to install Acquire^{MP}. Enter the license key into the Acquire^{MP} dialog and use the **Browse** button to select the .skm activation file you copied over, then click **OK**. Acquire^{MP} will now load.

Deactivating the license

If you wish to move your license for Acquire^{MP} to another PC, you should deactivate the license on the PC you no longer wish to use. To do this, open Acquire^{MP} and go to **Help** > **Deactivate license**. A warning message will be shown. Clicking **Yes** will close Acquire^{MP} and deactivate the license on that PC. You can then activate your license key on another PC. To reactivate a license after deactivating it, simply open Acquire^{MP} again and re-register the license, following the instructions above.

Check device status

After starting Acquire^{MP}, the status of each device in the Mass Photometer can be checked in **Tools > System Status…**. A notification will appear concerning whether any devices have failed to start correctly.

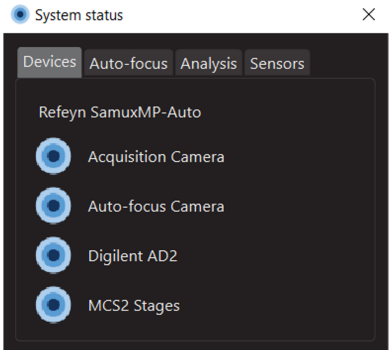


Figure 3: Dialog showing that all devices have started correctly for Two^{MP} and Samux^{MP}. The dialogue for the One^{MP} will also list the DAC

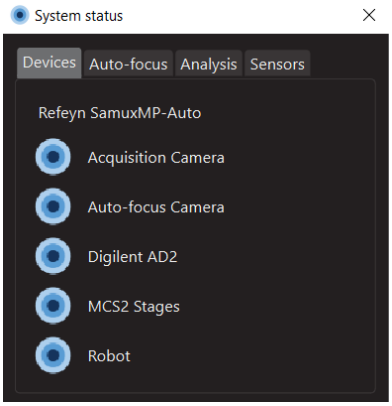


Figure 4: Dialog showing that all devices have started correctly, for Two^{MP} Auto and Samux^{MP} Auto. The dialogue for the One^{MP} Auto will also list the DAC

Acquisition workflow

The workflow necessary to acquire Mass Photometry data will depend on the Mass Photometer being used. Below, the main steps of the data acquisition workflows are described for the multiple devices supported by Acquire^{MP}. Further details on the different steps defined in the workflow are available in the following sections of this User Manual.

Refeyn One^{MP}, Two^{MP} and Samux^{MP}

1. Define measurement settings.
2. Position your cassette and find focus.
3. Load sample.
4. Record movie and save the directory of the measurement folder.

Refeyn One^{MP} Auto, Two^{MP} Auto and Samux^{MP} Auto

1. Define the well plate design and protocol and set-up the hardware.
2. Define measurement settings.
3. Align the cassette.
4. Run protocol and save the directory of the measurement folder.

A user can chose to add the well plate after defining the protocol to be run by the robot.

Functionality and hardware compatibility

This version of Acquire^{MP} is compatible with multiple Mass Photometers. Depending on the device being operated, different functionalities will be available. A summary of the functionalities available for each device is described in the table below. Further details are provided in following sections of this User Manual.

	One^{MP} & Two^{MP}	One^{MP} Auto & Two^{MP} Auto	Samux^{MP}	Samux^{MP} Auto
Normal Measurement Mode	X	X		
AAV Measurement Mode	X	X		
Samux Measurement Mode			X	X
Image Size	Multiple options	Multiple options	Fixed	Fixed
Movie Length	Customisable	1, 2 or 3min	Customisable	1, 2 or 3min
Droplet-Dilution Find Focus	X	X	X	X
Buffer-Free Find Focus	X	X	X	X
Manual Focus	X		X	
Manual Stage Control	X		X	
Automatic Cassette Alignment		X		X
Analysis Preview	X	X	X	X
Analyse During Protocol		X		X
Auto Robot Control		X		X
Advanced Settings	X	X		
Concentration Calculator	X	X	X	X
Hints and Tips	X	X	X	X

One^{MP}, Two^{MP} and Samux^{MP} workflow

Main window features

Toolbar

The toolbar contains many of the important settings required to optimise the recording as well as useful tools. Details on each of these are provided below.

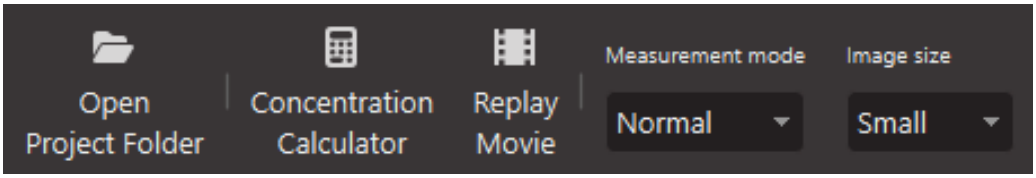


Figure 5: Toolbar. Note: Measurement mode and Image size controls are only visible on the One^{MP} and Two^{MP}

Acquisition visualisation

The acquisition camera is the primary camera of the Mass Photometer. It records the images that are used to measure the mass of particles that bind to (or unbind from) the slide. Images from the acquisition camera are displayed in the top half of the Acquire^{MP} main window.

Selecting the **Native** and **Ratiometric** buttons toggles between these views. The pixel brightness limits on the right-hand side depend on the viewing mode. Native image pixels have a brightness value between 0% and 100%. Selecting **Auto** automatically scales the applied color gradient between the values of the darkest and brightest pixel in each frame. Unchecking the **Auto** box allows users to change the values of the color gradient limits. If a pixel has a value less or greater than the respective minimum or maximum limits then it will be assigned the minimum or maximum color gradient value. In Ratiometric mode a change made to either limit is automatically mirrored in the opposite limit to ensure that both binding and unbinding events are proportional.

The recommendation is to ensure Auto is enabled in native mode.

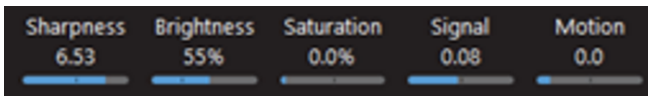
Figure 6: Acquisition movie information panel in Native mode

Data quality monitors

On the right side of the interface are five data quality monitors - Sharpness, Brightness, Saturation, Signal and Motion. These values should remain in the blue regions; if they enter the orange region, this indicates that the data being collected may be unreliable. The exception to this is that the signal monitor will become orange whenever a sample is added due to landing events increasing the signal.

- The **Sharpness** parameter relates to the clarity of the pattern in the acquisition image and is therefore the key to the mass photometer's ability to be able to discern landing events.
- The **Brightness** is the median illumination of all pixels in the acquisition image.

- The **Saturation** is the level of saturation of the acquisition image.
- The **Signal** monitor displays the root-mean-square (RMS) deviation of the ratiometric acquisition image.
- The **Motion** value gives an indication of how much lateral motion the sample is subjected to, either by vibration or by drift.



Autofocus

The bottom-left of the Acquire^{MP} main window displays the autofocus indicators. The mass photometer's auto-focus optics produce a circular ring image, shown on the left-hand side of the panel. The radius of this ring is directly related to the vertical (Z) position of the glass slide. Next to the ring image are two plots: the **Focus Position Monitor**, showing the ring radius over time, and the **Radial Profile Monitor**, showing the radial average of the ring image.

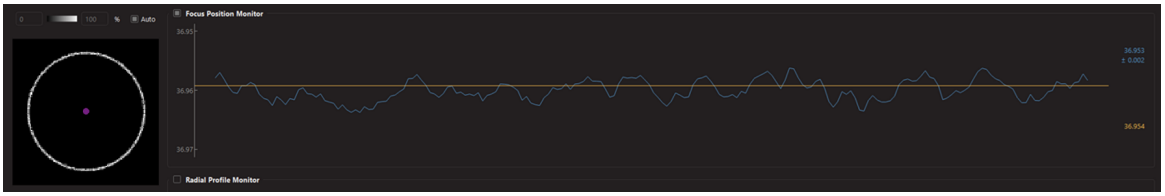


Figure 7: Focus position monitor and ring

Replay Movie

The **Replay Movie** dialog allows movies in the current project folder to be viewed. Movies are selected by clicking on the filename in the panel on the left of the window. Using the radio buttons, the view can be changed between **Native** and **Ratiometric**. The playback timeline can also be changed between seconds and frames using the dropdown in the bottom right.

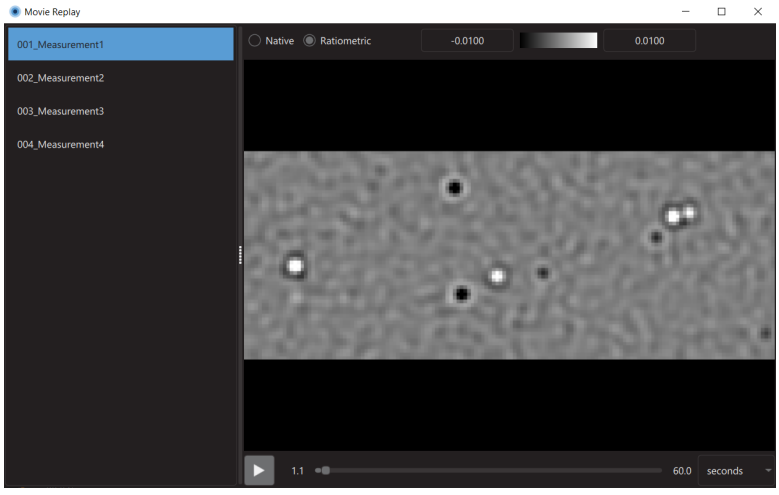


Figure 8: Replay movie window

Create, load and save projects

Projects are the way that Acquire^{MP} handles sample recordings and enables their playback.

To create a new project folder or open an existing one click **Open Project Folder** in toolbar. In the window that opens, create a new folder or select an existing one, and click ok. Alternatively, this can be done following the first recording.

Acquisition Options

Movie length

The length of the movie to be recorded can be set by editing the textbox positioned to the right of the movie slider. The value can be defined in seconds or frames, depending on the dropdown option selected next to the textbox.

Measurement Mode (Adjustable on One^{MP} and Two^{MP} only)

A particular measurement mode can be chosen depending on the type of measurement being performed. Different measurement modes will activate different analysis tools when the movie is loaded in Discover^{MP}.

- **Normal**: This mode gives the most flexibility and should be used for most measurements.
- **AAV**: This mode is for performing measurements to calculate the full/empty ratio of a sample of adeno-associated virus (AAV).

Users can choose between **Normal** and **AAV** mode using the dropdown in the tool bar. By default, Acquire^{MP} will use the Normal measurement mode. The Samux^{MP} and Samux^{MP} Auto only operate in **Samux** mode, optimised to calculate the full/empty ratio of a sample of adeno-associated virus (AAV).

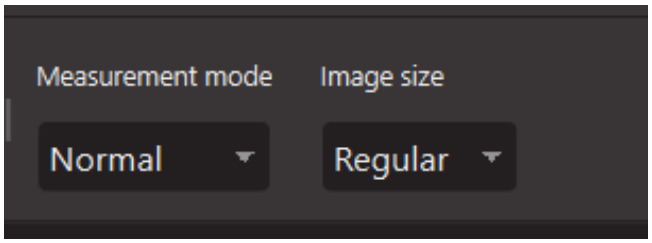


Figure 9: Measurement mode and image size controls

Image Size (Adjustable on One^{MP} and Two^{MP} only)

AAV and **Samux** measurement modes have a fixed large image size, while in the **Normal** measurement mode, the user may choose from three pre-defined image sizes.

On the One^{MP} these are *Regular*, *Medium*, and *Large*. *Regular* is the default setting.

On the Two^{MP} the sizes are called *Small*, *Regular*, and *Large*. *Regular* is the default.

For guidance on which image size is appropriate for your experiment, see **Image Size** in the **Further Information** section.

Positioning the cassette and focusing

Stage control

At the bottom right of the main display window is the lateral control tab, used to move the stage laterally and a focus control tab, containing manual focus functions. For more details on manual focus, see **Manual Focus** in the **Further Information** section.

The lateral movement of the stage is controlled using the arrows and the speed of the movement can be adjusted using the slider.

The stage can also be moved using keyboard shortcuts:

Command	Keyboard Shortcut
Up	Alt + W
Down	Alt + S
Left	Alt + A
Right	Alt + D

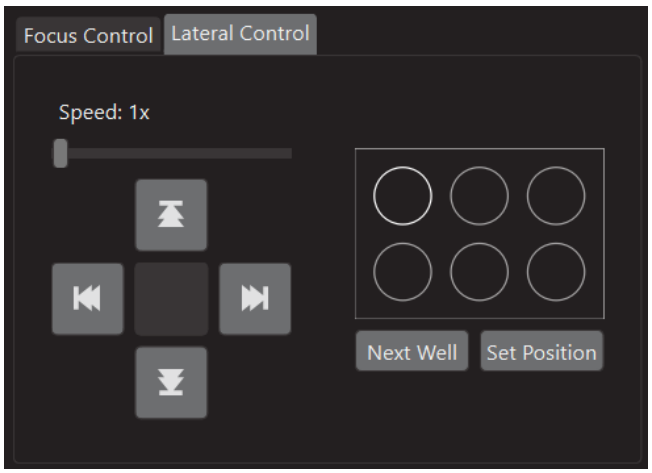


Figure 10: Lateral control tab with stage controls on the left and cassette navigation control on the right

Cassette navigation

First, drop some immersion oil onto the objective, and then place an assembled slide and cassette onto the stage, securing with magnets. In the cassette navigation control, double click on the well you wish to move to and the stage will move accordingly. Then using the arrows, position the well above the objective using the eye safe red laser. For best performance, slightly offset the laser from the centre of the well. Once positioned, click **Set Position**, and you will be able to move to the next well using the **Next well** button. You can also double click on the well you wish to use.

It is recommended to ensure the focus is unlocked before moving the stage laterally.

TIP The cassette navigation tool is only designed to work with pre-cut 6 well cassettes which are available from the Refeyn Web Shop.

Find focus

Acquire^{MP} has two **Find Focus** features that will automatically focus the system. These are:

- Droplet-Dilution Find Focus
- Buffer-Free Find Focus

Alternatively, a user can decide to use the manual focus option, the details of which can be found in the **Advanced Features** section of this manual. To focus, the Current Focus **Lock** must be unlocked. If it is locked (indicated by an orange closed padlock in the button), click the **Unlock** button to unlock.

TIP Measurements must use the same find focus routine as their respective calibrations.

TIP The instrument should be turned on 1 hour before use to allow time for thermal equilibration.

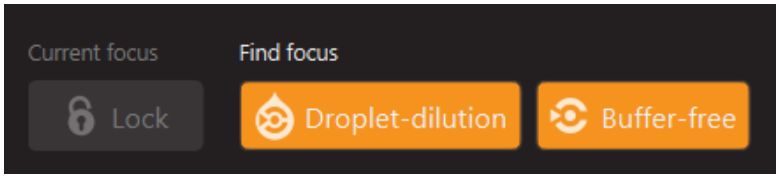


Figure 11: Controls for focus lock, Droplet-dilution find focus and Buffer-free find focus

Droplet-dilution find focus

Droplet-dilution find focus requires access to a particle free buffer which is the same as that used for your sample, but is the preferred method for low mass protein measurements.

To use droplet-dilution find focus, pipette 10–15 µl of particle free buffer into the well, close the lid and click **Droplet Dilution** in the find focus section, and the instrument will find focus.

When run for the first time, Find Focus usually takes up to 20 seconds to complete as it searches across the Mass Photometer's focusing range. On future runs, the previous focus position will be used to speed up the search. For the Find Focus routine to work effectively, the auto-focus and the acquisition cameras need to be set up to give a good image.

Verify the quality of the focus as instructed below before opening the lid, adding sample to make the final volume in the well up to 20 µl, closing the lid and clicking **Record**.

Buffer-free find focus

Buffer-free find focus provides a simplified faster measurement process.

To use Buffer-free find focus, ensure the well is centered on the objective, close the lid and click **Buffer-Free**. The system will perform a course focus and then ask that sample is added to the well. Pipette 20 µl of sample into the well, and close the lid. If the **Automatically detect if sample is added** checkbox is checked, the system will automatically complete the focussing process, otherwise, click **Sample Added**.

If the **Start recording automatically** checkbox is checked, then the recording will start automatically 6 seconds after the focus is complete. If not, then click the **Record** button to the right of the find focus buttons.

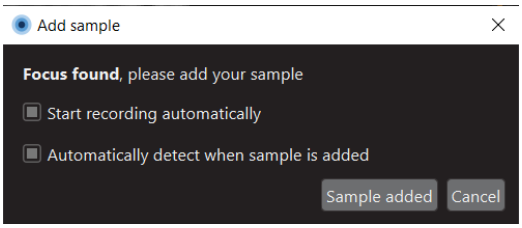


Figure 12: Dialog prompting the user to add sample during Buffer-Free Find Focus

Focus Quality

There are three indicators of focus quality in Acquire^{MP} software. These are:

1. Focus ring
2. Native image
3. Quality monitors

The focus ring in the bottom left of the main window should be reasonably round and have reasonably even contrast. If there are gaps, this is generally an indication of bubbles in the immersion oil. To remove these bubbles, unlock the focus, remove the magnets and lift one edge of the cassette assembly, breaking the immersion oil droplet before replacing the slide. Resecure using the magnets and repeat the focus procedure.

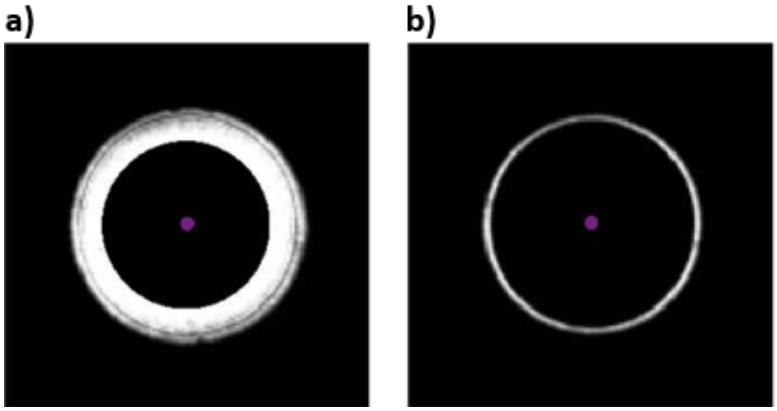


Figure 13: Ring images indicating a good focus a) with Buffer-free find focus before samples has been added, b) following the completion of either find focus method

The native image in the top left of the main window should show a sharp image of the roughness in the glass. Look around the image for the presence of large particles such as dust which will appear as large light or dark patches as shown below. To do this unlock the focus, move the stage until the particles are outside of the field of view, and repeat the focussing procedure.

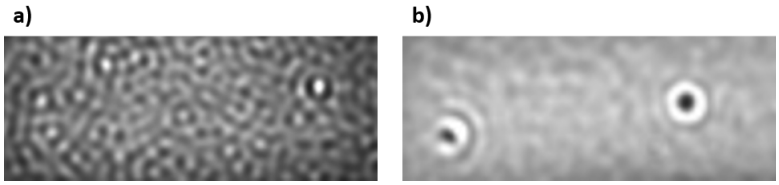


Figure 14: Dirt appears as large a) bright, or b) dark spots in the native image

The quality indicators are positioned in the centre right of the main window. After focus, these should all be blue. Once sample has been added, the signal indicator may move into the orange and this is completely normal.

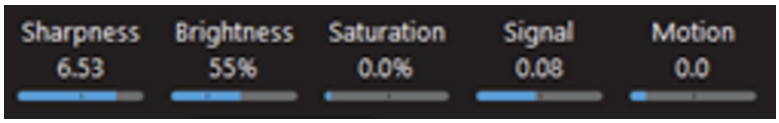


Figure 15: All quality indicators showing blue

Movie recording

Recording and saving

Once recording has begun (see Find Focus), the progress bar in the Acquisition Movie panel shows the movie progress. Acquire^{MP} will record up to the number of seconds or frames shown on the right of the progress bar. The movie will stop recording at the selected time or upon pressing the **Record** button again.

When the recording ends, a dialog will appear asking the user whether the recording should be saved, and what filename to save it as. The project for the measurement can also be defined here.

Additional information about the measurement can also be recorded so it can be viewed in Discover^{MP}. At the bottom of the dialog is a **Fill Previous Values** button, which fills the values from the previous measurement when clicked. This can be used to complete the fields when multiple recordings of the same sample are to be saved.

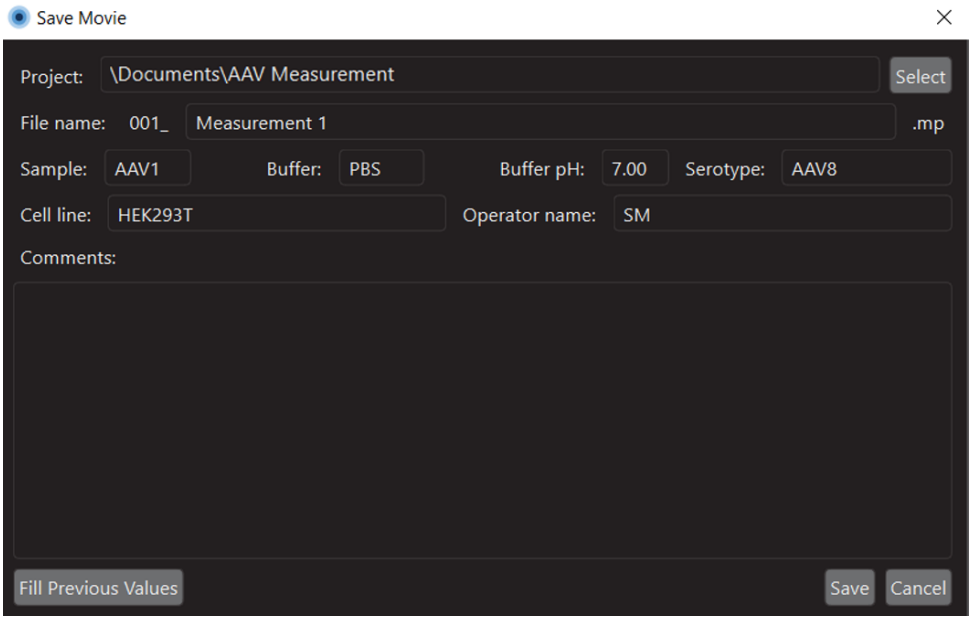


Figure 16: Save movie dialog for Samux^{MP}

By default, the saved recordings are compressed to save disk space and will be decompressed when being analysed in Discover^{MP}. Details on turning file compression on or off are detailed here.

Analysis preview

The top right of the Acquire^{MP} window displays the **Analysis Preview Panel**.

When enabled, this panel will show a preview of the data currently being acquired, in the form of a histogram. For larger image sizes, the histogram will only show a portion of the landing events detected. By default, the analysis preview histogram shows ratiometric contrast on its X axis.

This can be changed to show particle mass by loading a mass calibration (.mc) file created in Refeyn Discover^{MP}. To do this, right-click on the histogram and select **Load Mass Calibration**. On choosing a mass calibration file, the histogram will change to show mass on its X axis. To switch back to ratiometric contrast, right-click on the histogram and select **Clear Mass Calibration**.

Analysis

Now that the movie recording is complete, the movie can be imported and analysed in Discover^{MP}.

One^{MP} Auto, Two^{MP} Auto and Samux^{MP} Auto workflow

Main Window Features

The experiment designer is made up of 3 main elements:

- Toolbar - Located at the top of the window it contains the controls required to open and save experiment files, control the mass photometer and control the robot.
- Well plate panel - This is located on the left of an experiment tab and allows you to define the layout of the well plate for the experiment.
- Protocol panel - This is found on the right of the experiment tab and allows you to define the protocol steps in your experiment.

Toolbar

The toolbar contains the tools required to perform a set of measurements. It consists of:

- The **New** button opens a new tab with a blank experiment.
- The **Open** button opens the selected experiment file in a new tab.
- The **Save** button saves the contents of the well plate and protocol table to a pre-existing file if the experiment has previously been saved or ask for a file location to be specified if it has not. The arrow to the right of the **Save** button shows the **Save As...** button which will always prompt for a new file location to be specified. This is a useful way to create a new protocol from an existing one.
- The **Concentration calculator** is a useful tool for calculating required dilutions when the concentration of your stock solution is known. For more information see **Tools & Help**.
- The **Measurement Mode** selector allows the measurement modes to be changed between Normal and AAV modes (One^{MP} Auto and Two^{MP} Auto only)
- The **Image Size** selector allows the acquisition image size to be changed (One^{MP} Auto and Two^{MP} Auto in Normal mode only). For guidance on which image size is appropriate for your experiment, see **Image Size** in the **Further Information** section.
- The **Tiprack** section contains the controls related to the pipette tiprack. **Reset to A1** makes the robot start the run at the first tip in the rack and would generally be used when a full tiprack has been inserted. Select next allows you to change the starting pipette tip. By default the system will assume the tiprack has not been changed since the last measurement and start the protocol using the next tip position.
- The **Starting cassette well** control facilitates the selection of the first well in the cassette (not the well plate) to be used in the experiment. By default this returns to 1 at the start of each experiment assuming a new slide and cassette have been installed, but if this is not the case, the starting well can be changed here.
- The **Reset Robot** button moves the robot back to its home position.

Figure 17: Toolbar for One^{MP} Auto and Two^{MP} Auto

Start an experiment

To create a new experiment tab, click **New** in the toolbar. This will open up a new tab with a blank well plate panel and protocol panel. Alternatively, you can open a pre-existing experiment file in a new tab by clicking

Open and selecting the experiment file. Once created, experiments can be saved using the **Save** or **Save As…** buttons in the tool bar.

Populate the well plate panel

The next step is to populate the well plate panel with the samples, buffers and calibrants required for the protocol.

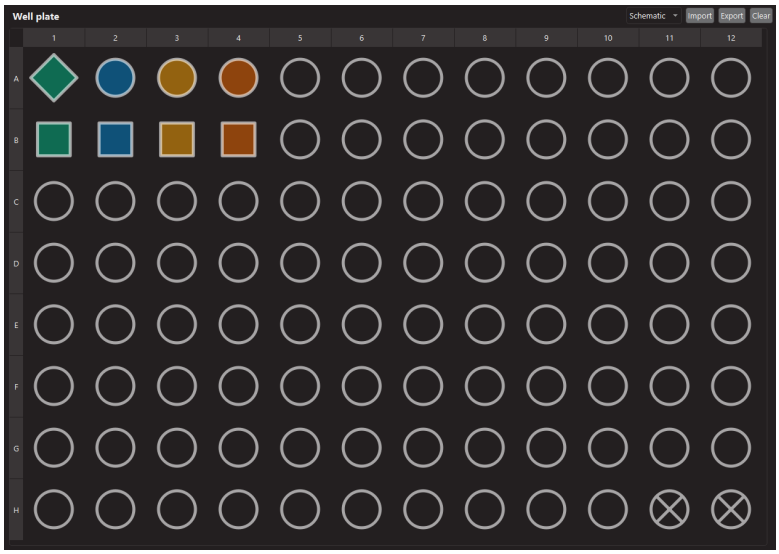


Figure 18: Well plate panel

Add a sample

Samples, buffers and calibrants are added to the well plate by double clicking on a well. The **Edit well** dialog can then be populated with the details of the sample.

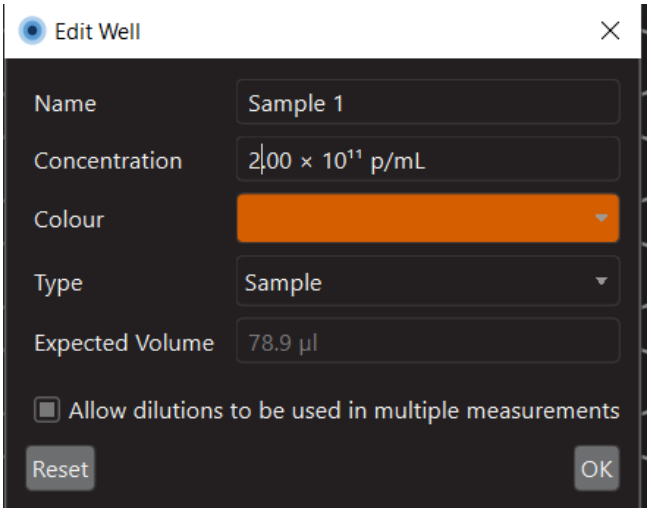


Figure 19: Edit well dialog

The window contains fields to record the **Name** and **Concentration** of the material which will be dispensed into that well. In addition, a colour can also be specified. This colour has no bearing on the experiment, but

can be used to help identify different groups of samples and buffers in both the well plate and protocol table.

The material **Type** should also be defined as either **Sample**, **Buffer** or **Calibrant**. This identification will be used by Discover^{MP} when analysing your data. To aid in the identification of these material types, the shape of wells will be changed. Circles are samples, squares are used for buffers and rotated squares for calibrants.

The **Expected Volume** field will be populated automatically, once the protocol table has been completed. This is the minimum amount of sample which must be dispensed into the well for the protocol to run successfully.

Finally, if the **Allow dilutions to be used in multiple measurements** checkbox is selected for the sample well, the robot will perform a single dilution (where requested) and use the same diluted sample for repeats of this measurement defined in the protocol table. If this is not selected, a new dilution will be performed for each individual measurement.

Import and Export

If there is a pre-existing well plate design you would like to use, you can use the **Import** command to populate the well plate from either a JSON file or a CSV file. Well plate designs can also be exported as a JSON or CSV for future use using the **Export** button.

Other well plate functions

In addition to those listed above, there are other tools to aid populating the well plate. At the top right of the well plate panel there is the **clear** button which clears the contents of the well plate. By right clicking on a well, you can:

- **Clear** the contents of a well. This can also be done by left clicking on the well and pressing the delete key.
- **Copy** the contents of a well so that you can **Paste** it into a new location. This can also be done by left clicking and dragging a well onto another well.
- **Edit** is another way to access the Edit Well dialogue.
- **Assign well as dirty** changes the colour of the well to mark it as dirty. This is useful if a partially used well plate is being used for an experiment.

All of these functions, other than edit, can be applied to multiple wells by holding the **Ctrl** key and left clicking on multiple wells. Holding **Shift** and left clicking on two wells will select the wells between those two.

Populate the protocol table

The next step is to populate the protocol table. To add a new step to the protocol, click the **Add** button below the table. Steps can also be removed and duplicated by clicking on a row and clicking **Remove** or **Duplicate** as appropriate.

Add protocol steps

Once a step has been added, a **Sample** to measure must be defined. This is done by clicking and dragging the well which contains the sample you would like to measure onto the sample cell of the appropriate row in the protocol table. If you wish to focus using droplet dilution, or want to dilute the sample as part of the protocol, a **Buffer** must also be specified by clicking on the relevant buffer well, and dragging it to the buffer cell on the relevant row of the protocol. The **Sample Volume**, and if required, a **Buffer Volume** must also be defined. If the buffer volume is set to >0, Droplet-Dilution Find Focus will be used for the measurement. If the buffer volume is set to 0, Buffer-Free Find Focus will be used. Volumes should be set as follows:

14 Well Cassette

Focus Method	Sample Volume	Buffer Volume	Total Volume
Droplet-Dilution Find Focus	5–10 µl	10–15 µl	20 µl
Buffer-Free Find Focus	10–20 µl	0 µl	10–20 µl

24 Well Cassette

Focus Method	Sample Volume	Buffer Volume	Total Volume
Droplet-Dilution Find Focus	5 µl	5 µl	10 µl
Buffer-Free Find Focus	5–10 µl	0 µl	5–10 µl

The next step is to set the **Sample Dilution**. This feature allows samples to be diluted by up to 1/1000. Simply click on the cell and use the drop down menu to select a dilution. To use this feature, make sure a buffer is defined in the protocol step. As dilutions are added, wells will appear with a white cross in the well plate panel. These wells are reserved by the system to use to create dilutions.

When using Droplet-Dilution Find Focus, remember that the sample will be diluted further by the buffer in the cassette well.

Measurements must use the same find focus routine as their respective calibrations.

The **# Runs** column determines the number of times each step in the protocol is repeated and **Movie Length** can be adjusted to 1, 2 or 3 minutes. Generally speaking, a 1 minute movie is recommended. If you would like all measurements to have a length of 2 or 3 minutes, we recommend changing one measurement and duplicating it.

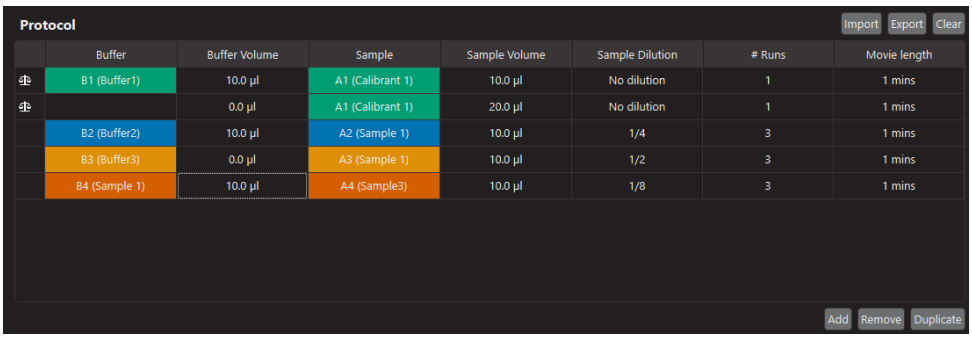


Figure 20: Protocol table

Import and Export

If there is a pre-existing protocol table you would like to use, you can use the **Import** command to populate the protocol table from a CSV file. You can also **Export** protocol tables as a CSV file for future use using the **Export** button.

Filling Wells

Once the well plate and protocol have been inputted into the software, we recommend using the table view of the well plate to check the minimum volumes of sample or buffer required in each well. Switching the well plate to table view is done by clicking on the dropdown above the well plate design, which can switch between **Table** and **Schematic**.

Define Run Settings

Before running a protocol, it is important to check the settings in the toolbar. First, on One^{MP} Auto and Two^{MP} Auto only, the **Measurement Mode** and **Image Size** should be set using the drop down.

The user can choose a particular measurement mode depending on the type of measurement they are performing. Not all measurement modes are available for all Mass Photometry devices. Different measurement modes will activate different analysis tools when loaded in Discover^{MP}.

- **Normal**: This mode gives the most flexibility and should be used for most measurements.
- **AAV**: This mode is for performing measurements to calculate the full/empty ratio of a sample of adeno-associated virus (AAV).

For more details on choosing an image size, see the **Image Size** section under **Further Information**.

Next, the starting conditions in the robot should be set:

Tiprack: The software will remember the position of the last tip it picked up in the previous run and will continue from the next position. The software will assume that all subsequent positions on the tiprack contain a tip. When adding a new tiprack, users can reset the software by selecting **Reset to A1**. If you would like to start from a different tip, click **Select next** and a dialog will appear allowing you to select the starting tip by clicking on the starting position and clicking **Select tip** to confirm. The robot will pick up tips column by column from top to bottom. ie. A1»A12, B1»B12…H1»H12

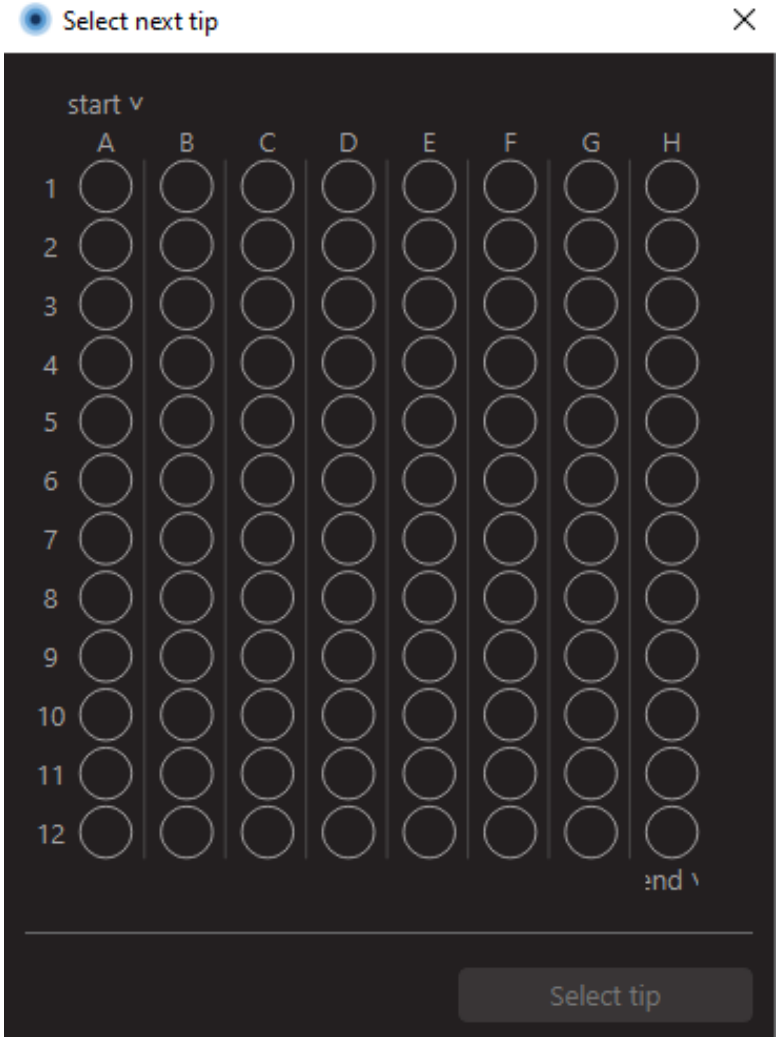


Figure 21: Dialog to select next pipette tip to use

Starting cassette well: For the starting cassette well, the software will normally begin with cassette well 1 (top left position). However, you can input a different starting cassette well if necessary. This can be done by changing the value in the **Starting Cassette Well** control. Note that the well order snakes rather than running only from left to right.

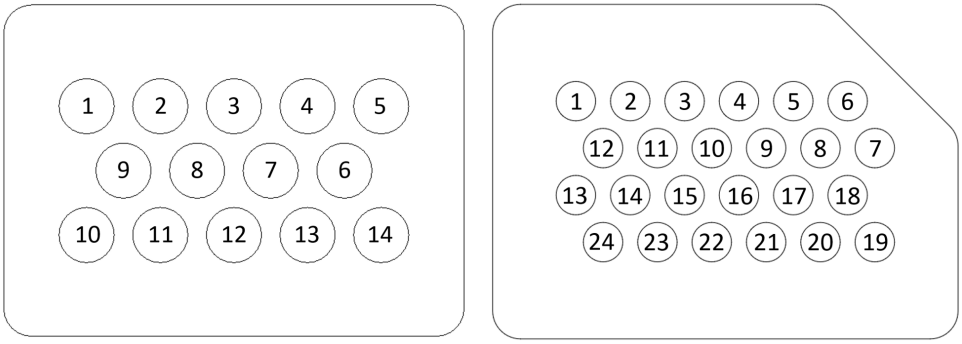


Figure 22: 14 and 24 well cassette layout as seen from above the Mass Photometer stage

Choose When to Run Analysis

You can choose for the system to analyse the data as it is being recorded by checking the **Analyse during protocol** box in the bottom right of the main window. Alternatively, you can analyse the data afterwards in Discover^{MP}. Analysing whilst recording is the quickest way to get analysed data from your measurements but if you want the protocol to run as quickly as possible, data should be analysed afterwards. This could be the case if you have samples prone to aggregation over time.

Align Cassette

Before any run, the cassette must be aligned. This is done after oil has been deposited onto the objective and an assembled clean slide and cassette have been placed onto the stage and secured with magnets. The alignment process will use two wells to map the locations of the wells on the cassette. The easiest way to do this is to click **Align cassette** in the bottom right hand corner. This will automatically run the alignment process.

When assembling the cassette onto a clean slide, make sure to use the aluminium alignment tool provided by Refeyn to ensure the Align Cassette routine works reliably.

In the event that automatic alignment fails, a manual alignment option is available in **Settings > Cassette Settings…** and instructions on how to use this are located in the **Further Information** section of this document.

Run Protocol

Once the cassette has been aligned and the well plate, tiprack and waste tip collection box are present you are ready to run the protocol. To do this, click **Run** in the bottom right corner of the tab. This will open a dialog prompting you to select a folder for the data to be saved to.

If there are any errors or warnings, these will be flagged in a pop-up as shown below.

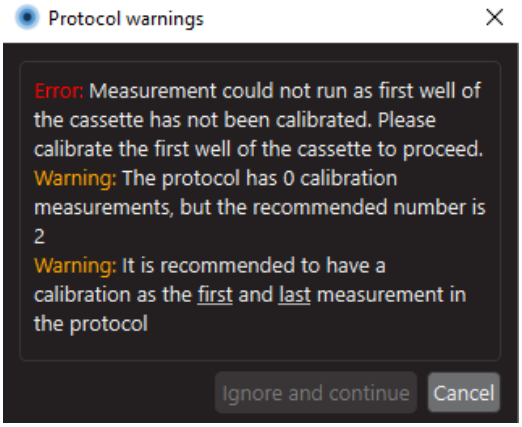


Figure 23: Error and warning example

Finally, a window will appear asking you to confirm that the well plate layout is correct. Once this is confirmed the protocol will begin to run.

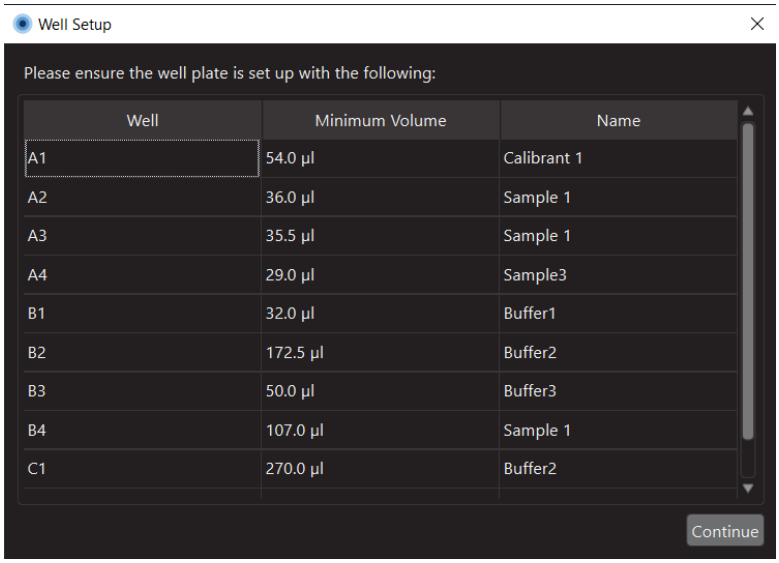


Figure 24: Dialogue confirming the well plate layout

Monitor Progress

Once the run has begun, the tab will show the **Progress monitor**, which is made up of 4 sections:

- **Acquisition camera image** - Displays the live ratiometric or native image.
- **Analysis preview** - Gives an indication of the measurement results. This is not the same as the analysed data which can be viewed in Discover^{MP}.
- **Progress details** - This provides details on the run progress, highlighting any errors or warnings that may have occurred.
- **Progress bar** - Displays an estimate of progress through the protocol run.

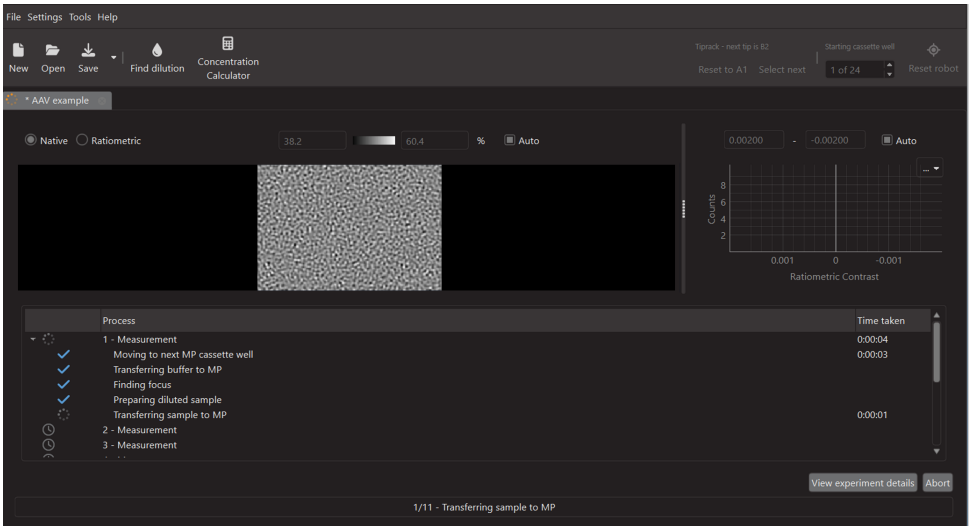


Figure 25: Progress monitor layout

In addition, you can **Abort** the protocol run and **View experiment details** in a pop-up window using the buttons in the bottom right. You can also start creating your next experiment by selecting or opening a new tab.

If the experiment has been halted due to an unexpected error, the robot arm may become stationary in its last operating position. Use the **Reset Robot** button to position the arm of the robot in the resting position.

Analysis

If you chose to analyse whilst the protocol was running then your analysed data can now be visualised in Discover^{MP}. If your data still needs to be analysed, then this must be done in Discover^{MP}.

To return to the experiment design, click **Back to experiment** in the bottom right.

Tools & Help

Concentration calculator

The concentration calculator allows users to calculate the volumes of reagent and solvent needed to dilute a solution to a particular concentration. It also allows users to convert between mass concentration and molar concentration given a molecular weight. The concentration calculator can be opened from the **Tools** menu.

To calculate the volumes of reagent and solvent needed for a dilution, enter the current and target concentration of the solution into the corresponding fields in the dialog. The dialog will display the dilution factor and, once a target volume has been entered, the required reagent and solvent volumes. If the calculation requires a molecular weight, due to conversions between mass and molar concentrations, a warning message will appear prompting the user to complete the molecular weight field.

To convert between mass and molar concentrations, enter the concentration into the current concentration field, adjusting the units as required. Then enter the molecular weight, and the converted concentration will be shown below the current concentration field, in the units specified by the dropdown to the right.

The dialog will show a warning if the dilution is too large (indicating that the units may be incorrect), or if the target concentration is too low to be detected by the Mass Photometer.

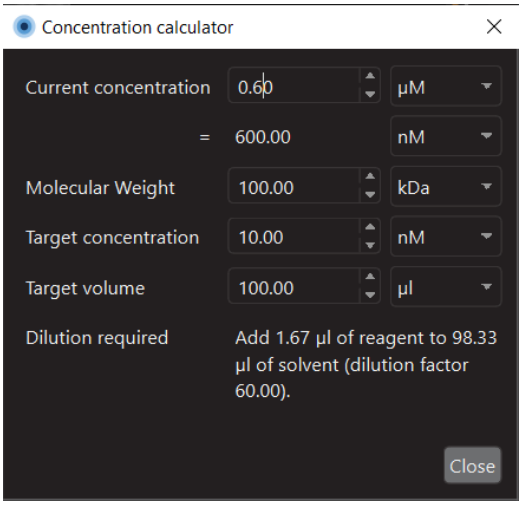


Figure 26: Concentration calculator

Hints and Tips

Upon startup of Acquire^{MP}, a dialog will appear with hints and tips on how to perform a normal mass photometry measurement. These tips are intended to guide new users though the measurement process and are not a replacement for proper training. The dialog can be disabled by unchecking **Show tips at startup** in the bottom-left corner. The dialog can be re-enabled by opening the dialog from the **Help** menu, and checking the box again.

The dialog presents each step of the measurement process as a separate page. The user can advance to the next step using the **Next** button, or by selecting a step using the list on the left. The main Acquire^{MP} interface remains active while the dialog is open, so users can perform each step with the tips dialog open. Some steps include links to external resources, such as video tutorials.

Figure 27: Startup tips dialog

Further Information

Image Size

Increasing the image size means that more events can be detected in a single frame which results in improved statistics. However, changing to a larger image size lowers the mass sensitivity. This is due to the same amount of light being distributed over a larger area, decreasing the per-pixel intensity and signal-to-noise ratio. The increased noise is reflected by a grainier ratiometric image and a higher signal score when measuring clean buffer. Increasing the image size also increases the file sizes and processing times.

Ultimately, the optimal image size is completely sample dependent, but the limit of detection using the *large* field of view on the One^{MP} and Two^{MP} is 100 kDa and 70 kDa respectively. Ensure that the smallest population in a sample is significantly larger than this before moving to the *large* image size.

Note that the small image size is included for legacy reasons on the Two^{MP} as there is no detection improvement between the *small* and *regular*; hence, we recommend *regular* is used for low mass samples.

Note that the detection area does not exactly match the total area of the acquisition image because a border mask is applied during analysis to exclude peaks partly outside the image.

Image size data table for the One^{MP} and One^{MP} Auto

Image size	Binned pixels	Dimensions of imaged area	Detection area
Regular	128 × 34	10.8 µm × 2.9 µm	18.0 µm^2
Medium	128 × 81	10.8 µm × 6.8 µm	56.4 µm^2
Large	128 × 128	10.8 µm × 10.8 µm	95.0 µm^2

Image size data table for the Two^{MP} and Two^{MP} Auto

Image size	Binned pixels	Dimensions of imaged area	Detection area
Small	150 × 38	10.9 µm × 2.7 µm	29.8 µm^2
Regular	150 × 59	10.9 µm × 4.3 µm	46.3 µm^2
Large	233 × 166	16.9 µm × 12.0 µm	202.4 µm^2

Image size data table for the Samux^{MP} and Samux^{MP} Auto

Image size	Binned pixels	Dimensions of imaged area	Detection area
Samux^{MP}	233 × 166	16.9 µm × 12.0 µm	202.4 µm^2

Manual Focus

Find focus

The user also has the option to find the focus manually. This feature is only available when using the One^{MP}, Two^{MP} or Samux^{MP}. To perform a manual find focus, a droplet of buffer must be placed in the measurement well as would be done for a Droplet-Dilution Find Focus. The user must then use the coarse focus controls to reach a point where the roughness of the glass surface can be seen in the acquisition image and simultaneously a bright ring is visible in the auto-focus image. Once the ring is apparent, the fine focus controls can be used to bring the sharpness of the acquisition image to an acceptable level.

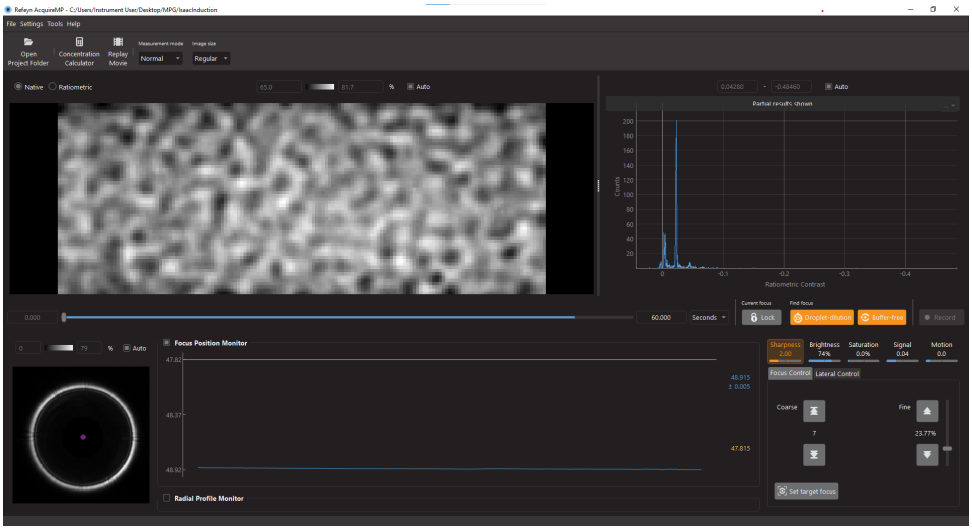


Figure 28: Mass photometer close to focus; both the auto-focus ring and glass roughness are visible

The data quality monitors above the focus control panel displays the values for sharpness, saturation, signal and motion which are considered acceptable when the Mass Photometer is in focus. The sharpness needs to be brought into the acceptable range using the Fine Focus controls. The optimal focus is achieved when the sharpness is greatest. Clicking on the **Sharpness** indicator displays a plot showing its value over time. Figure 29 shows an example of how the sharpness changes as the slide moves in the Z direction; it shows a noticeable maximum sharpness peak.

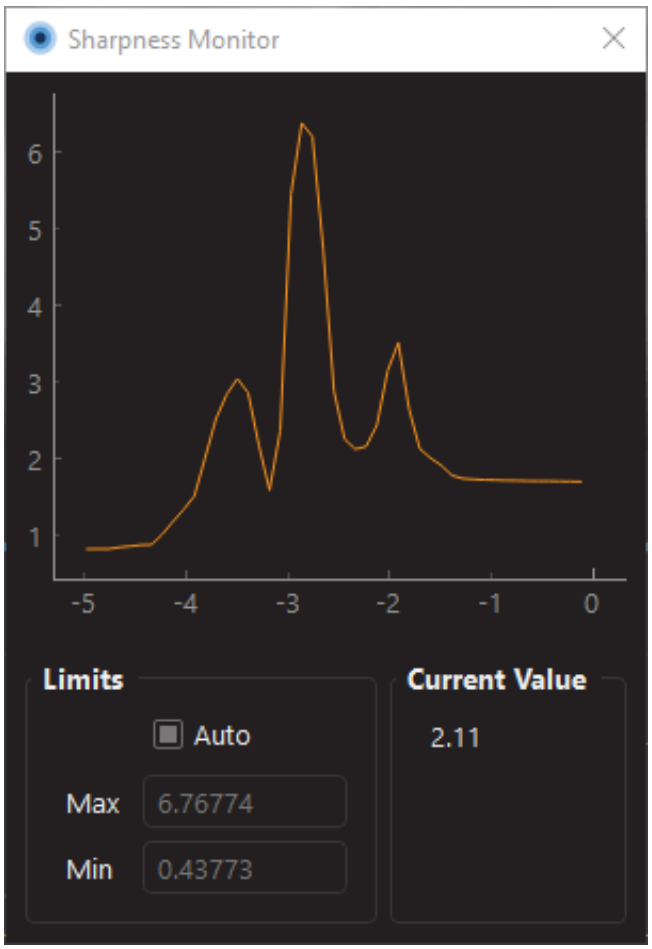


Figure 29: Finding the maximum sharpness value

When the instrument is in optimal focus the acquisition camera image in native mode will clearly display the glass surface (see Figure 30).

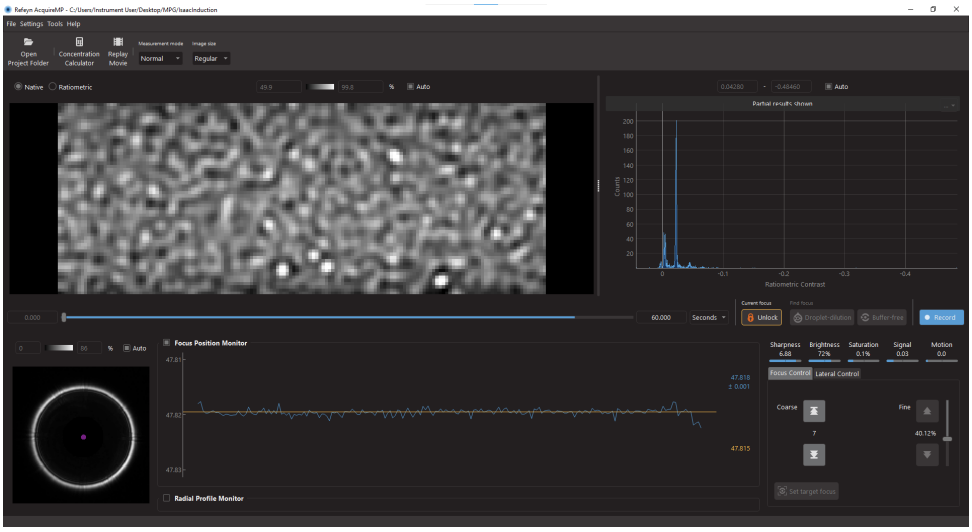


Figure 30: Acquire^{MP} with the mass photometer in optimal focus

Data quality monitors can give a good indication if focus has been found:

- If the Brightness value is too low, there is not enough illumination across the Acquisition image and the exposure time should be increased.
- The Saturation can be adjusted by modifying the exposure time of the acquisition image. The ideal is to have the exposure time as high as possible while keeping saturation at 0%. This is optimised using the **Calibrate Acquisition Image** tool which is accessed by clicking **Settings > Acquisition…**.
- The Signal parameter should be as low as possible in order to observe landing events of small proteins. If the signal is too high (slider turns orange), the recommendation is to move to a cleaner area of the well or to replace the slide.
- The Motion value should be minimised by isolating the Mass Photometer from external sources of vibration, making sure that it is mounted horizontally and using the provided magnets to secure the slide on the sample carrier.

In certain cases, drift is observed shortly after the position of the sample carrier is changed. For best results, it is recommended to wait until the position of the sample carrier has reached an equilibrium before recording is commenced.

Stabilise focus

Once the position of the optimal focus has been achieved and equilibrated (equilibration time is about one second), selecting the **Set Focus** button saves the auto-focus ring radius as a reference point for achieving a focused image.

The target ring radius is shown as a orange horizontal line in the **Focus Position Monitor** graph. The measured radius of the auto-focus ring is shown updating in real-time as a blue line.

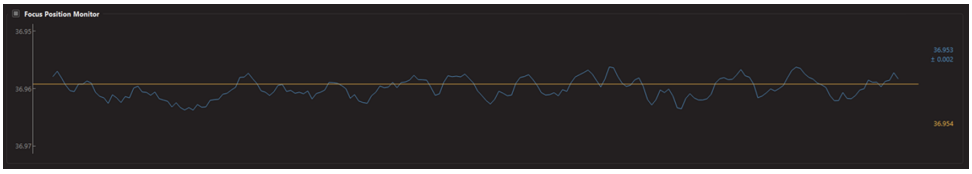


Figure 31: Focus position monitor when a target radius has been set

The current focus **Lock** button becomes enabled once a target radius has been set. On selecting Lock Focus, the Acquire^{MP} application enters Focus Stabilising mode; the sample stage is moved in the focusing plane to maintain a constant auto-focus ring diameter. In this mode, the Mass Photometer dynamically counteracts any drift in the focal position of the sample. The current focus **Lock** button displays a red unlock icon while in Focus Stabilising mode.

While the focus is locked, manual control of the sample stage is disabled and the auto-focus Settings and Calibration may not be updated. It is also not possible to load a previously saved settings file.

To re-enable these functions, exit Focus Stabilising mode by again selecting the current focus **Unlock** button.

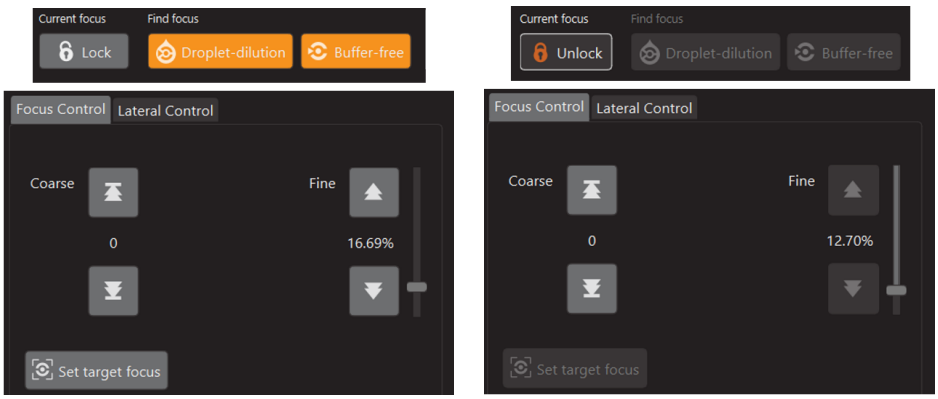


Figure 32: Lock Focus button enabled (left) and locked (right)

Manual Cassette Alignment

To perform a manual cassette alignment on a One^{MP} Auto, Two^{MP} Auto or Samux^{MP} Auto go to **Settings > Cassette Settings…** which will open the cassette settings dialog.

To perform a manual alignment:

1. Click **Go to first calibration well**.
2. Use the arrows to move the stage so that the highlighted well centered on the objective.
3. Click **Validate Position**.
4. Click **Go to second calibration well**.
5. Use the arrows to move the stage so that the highlighted well centered on the objective.
6. Click **Validate Position**.

The calibration is then saved.

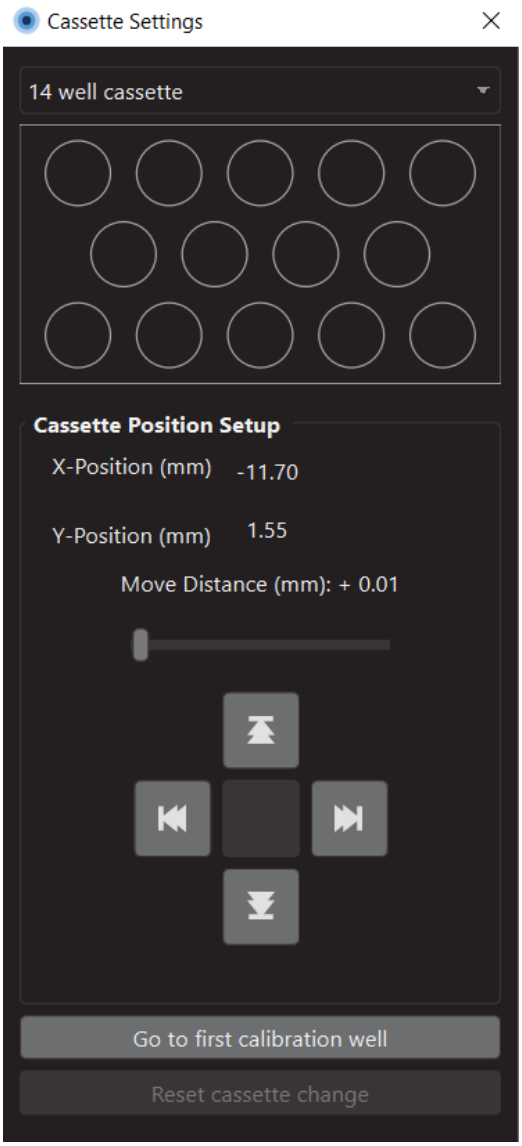


Figure 33: Cassette Settings window with tools for manual cassette alignment and for Two^{MP} Auto only, changing the cassette type

For the Two^{MP} Auto there is the option to change cassette type using the dropdown menu at the top of the window. If the type is changed a manual alignment is required to save the change and therefore, it is important to set up a slide with the new cassette type on the stage first.

Instrument Settings

Preferences

Acquire^{MP} has a small set of preferences that the user can interact with via **File > Preferences**.

By default, saved acquisition movies are compressed to reduce memory usage. When loaded in Discover^{MP} the movies are decompressed before being analysed. This compression can be turned on or off by checking or unchecking the **Enable file compression** option in the preferences dialog.

To show advanced settings (not available for Samux^{MP} and Samux^{MP} Auto) the user can press the **Advanced mode** button. This will bring up a message detailing the risks that can occur if the advanced settings are changed. The user will be questioned whether they would like to continue. To hide advanced settings, the user shall press **Leave advanced mode**. This option is not saved across sessions and by default will be turned off each time the software is started. Changes to the advanced settings can severely damage data acquisitions and analysis and should only be used when advised by Refeyn internal teams.

If the **Send usage data** box is ticked, Acquire^{MP} will report basic data about the user's use of the software to Refeyn, including error reports.

Save and load device settings

Acquire^{MP} handles projects and settings separately as this allows movies within the same project to have different instrument settings.

On startup, Acquire^{MP} remembers the most recently saved system settings. If Acquire^{MP} is being run for the first time, it will load the factory settings, or if there are none, the default settings. Pressing **Save Settings** updates the system settings, which will be loaded next time Acquire^{MP} is run. Pressing **Settings » Reset Settings** reverts the settings to the default. Acquire^{MP} can also save settings to a file to be reloaded later. This is done by going to **Settings** > **Save Settings As** and then choosing the location to save the current settings. **Settings** > **Load Settings** reloads a previously saved settings file. Settings files will affect all different settings defined under the Settings menu.

After running Acquire^{MP} for the first time, settings changes should not be required for most data acquisition experiments.

Troubleshooting

Calibrate Acquisition Image

This command should not be performed as part of a regular protocol and should only be used when the glass surface is not brightly or uniformly illuminated. To calibrate the acquisition image, open the acquisition settings dialog (**Settings > Acquisition…**) and click **Calibrate Acquisition Image**. This process takes approximately two minutes, and will optimise the settings for each image size simultaneously.

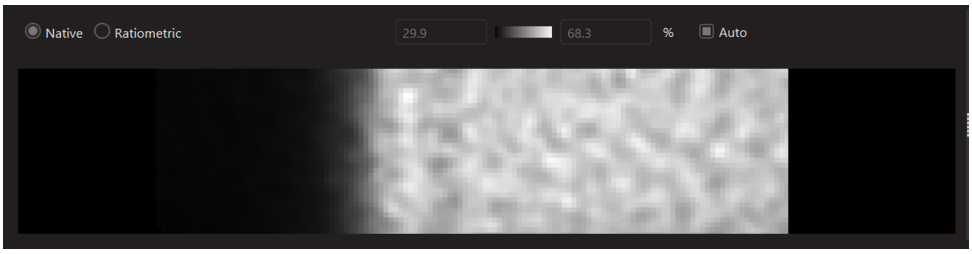


Figure 34: Partial illumination in the acquisition image which requires the acquisition image to be calibrated

Auto-Focus Ring Issues

Auto-Focus Settings can be found on the **Settings Menu** under **Auto-Focus**

Image parameters relate to reducing noise and masking out unwanted image reflections in the centre of the ring. The auto-focus mask should be in the centre of the observed ring when the acquisition image is in focus and there should be a few pixels of margin between the ring and the mask. To correct the position, change the image offsets, by checking the box **Show the Auto-Focus Mask** and then adjusting the **Horizontal** and **Vertical Offsets** to realign the ring. The **Mask Radius** sets the size of the mask to be applied in the centre of the auto-focus image. Whilst the auto-focus settings dialog is open the auto-focus mask will be highlighted to allow for easier adjustment. The **Noise Threshold** parameter applies a threshold to all pixel values, reducing their significance if they are below the threshold value.

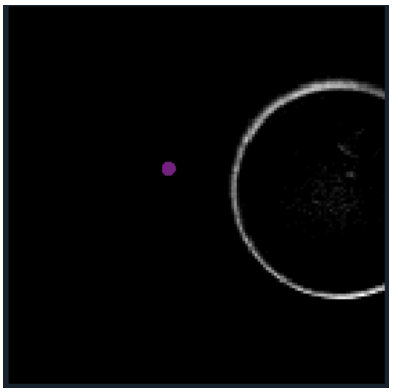


Figure 35: Off-centre auto-focus ring

Changing the **exposure time** alters the integration time of the auto-focus camera's chip for a single frame without altering the frame rate. If the camera's image is saturated, with the maximum intensity level at 100%, the user is advised to reduce the exposure time. Similarly, if the image appears underexposed, the exposure time should be increased to maximise the sensitivity of the instrument. A maximum intensity level of 95% is a good target for the auto-focus ring. The recommendation to ensure an optimal exposure time is to select the **Calibrate Auto-Focus Image** button in the Auto-focus settings menu.

The **Radial Profile Noise Threshold** parameter reduces the significance of radial positions with values below the threshold. This threshold is displayed by a yellow line on the Radial Profile monitor graph.

The **Calibrate Focus Position Stabiliser** option, enables users to accurately find and stabilise the focus position in Acquire^{MP}. After pressing **Calibrate Focus Position Stabiliser**, a calibration dialog will appear showing the parameters for the calibration process and the calibration currently used by the Acquire^{MP} software. This procedure determines the relationship between stage movements and the auto-focus ring radius. The calibration is carried out in the factory and usually does not need to be repeated by the user.

Only recalibrate the auto-focus if the factory settings are lost or if any physical component of the auto-focus system has been changed, such as the optical alignment or the set-up of the piezo sample stages.

Before calibration can be carried out, a slide with a droplet of buffer should be added and the Mass Photometer brought to the focus position. The auto-focus ring should remain visible during the entire calibration procedure. Figure 30 shows an example of when the instrument is the correct state to be calibrated as the auto-focus ring is clearly visible.

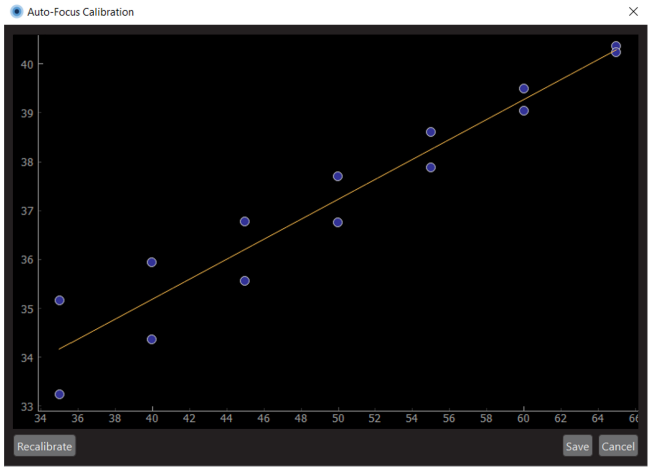


Figure 36: Calibrate Focus Position Stabiliser dialog showing completed calibration

The **Calibrate Auto-Focus Image** button will adjust the exposure of the image to give a good ring illumination.

The **Restore Defaults** will restore Auto-Focus camera settings to the factory settings if available, or the default settings.

The **Show Auto-Focus Mask** checkbox enables the highlight of the auto-focus ring mask region.

General information

Refeyn Ltd service and support

Refeyn Ltd offer service and technical support for our Mass Photometry systems, and the Refeyn Acquire^{MP} and Refeyn Discover^{MP} software applications.

Applications and service support

Refeyn Ltd
Unit 9, Trade City
Sandy Lane West
Oxford
OX4 6FF
Email: support@refeyn.com
Call: +44 1865 800175

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